Is inhibition of cancer angiogenesis and growth by paclitaxel schedule dependent?

Derick Lau[a], Linlang Guo[a], David Gandara[a], Lawrence J. T. Young[b] and Ling Xue[c]

It has been speculated that weekly paclitaxel enhances antiangiogenesis and, hence, results in a greater inhibition of cancer growth than the 3-week schedule. We compared the weekly and 3-week schedules of paclitaxel in inhibiting angiogenesis, tumor growth and bone marrow hematopoiesis in a lung cancer model. Vehicle or paclitaxel was administered i.p. to three groups of nude mice bearing a human lung cancer. The vehicle was given weekly for six doses or every 3 weeks for two doses (Group A). Paclitaxel was administered at 20 mg/kg/week for six doses (Group B) or 60 mg/kg/3 weeks for two doses (Group C). The tumor growth rate was reduced by 50% equally in both the paclitaxel-treated groups. Intratumoral microvasculature was reduced by 70% in each paclitaxel-treated group. However, white blood cell count was significantly reduced in Group C in comparison with that of Group A or B. We conclude that in this model, angiogenesis and tumor growth were inhibited to the same extent when paclitaxel was administered on a weekly or 3-week schedule. Inhibition of tumor growth by paclitaxel was associated with suppression of angiogenesis. Weekly administration of paclitaxel resulted in a lower degree of leukopenia than with the 3-week schedule, mimicking the clinical setting. *Anti-Cancer Drugs* 15:871–875 © 2004 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2004, **15**:871–875

Keywords: antiangiogenesis, dosing schedules, paclitaxel

[a]University of California, Davis Cancer Center and Veteran Administration Northern California Health System, Sacramento, CA, USA, [b]Center for Comparative Medicine, University of California, Davis, CA, USA and [c]Sun Yet Sen University of Medical Sciences, Guangzhou, China.

Sponsorship: This study was supported in part by Bristol-Myers Squibb Co.

Data from this study were presented in part at the Annual Meeting of the American Society of Clinical Oncology, 2001.

Correspondence to D. Lau, University of California, Davis Cancer Center, 4501 X Street, Sacramento, CA 95817, USA.
Tel: +1 916 734-3772; fax: +1 916-734-7946;
e-mail: derick.lau@ucdmc.ucdavis.edu

Received 13 April 2004 **Revised form accepted** 4 June 2004

Introduction

Paclitaxel (Taxol), a diterpene extracted from the bark of the Pacific yew tree (*Taxus brevifolia*), is one of the most active anticancer drugs in the treatment of breast, lung and ovarian cancers [1–3]. Although paclitaxel has been widely used in the clinic, the optimal dosage and schedule of administration of this drug have been debated, and are the subject of recent and ongoing clinical trials [3,4]. It has become a standard practice to administer paclitaxel as a 3-h infusion, either as a single agent or in combination with other cytotoxic agents, at doses of 175–225 mg/m^2 in 21-day cycles. However, phase I/II clinical trials have shown that paclitaxel, given weekly at 60–175 mg/m^2, is effective in inducing responses in breast and lung cancers, even in those refractory to paclitaxel given by the 3-week schedule [5,6]. Weekly paclitaxel has been reported to be better tolerated without compromising its anticancer activity. In addition, it is believed that, when given at low doses and frequent intervals, anticancer drugs exert antiangiogenic effects that may enhance anticancer efficacy [7]. For these reasons, weekly paclitaxel has been incorporated into recent and current randomized clinical trials for lung and breast cancers [8–10].

Paclitaxel exerts its cytotoxic action by inhibiting microtubule depolymerization [2,11]. Laboratory studies have shown that paclitaxel also inhibits neovascularization of chick allantoic membrane [13,14] and vascular endothelial growth factor-treated cornea in mice [15]. We previously demonstrated that paclitaxel exerted antiangiogenic effects at doses below that required for growth inhibition in a transgenic breast cancer model [16]. Furthermore, we have established a human lung cancer model and a method for quantitative analysis of intratumoral angiogenesis using confocal laser scanning microscopy [17]. To evaluate potential schedule-dependent effects of paclitaxel on angiogenesis, tumor growth and bone marrow hematopoiesis, we compared 7- and 21-day schedules in a lung cancer model.

Materials and methods

Lung cancer model

A model of adenocarcinoma of the lung was used as previously described [17]. A lung adenocarcinoma cell line, A549, was obtained from the ATCC (Manassas, VA) and maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO, Grand Island, NY). Cells were harvested with trypsin/EDTA, washed and

0959-4973

suspended in normal saline (NS) at a concentration of 10^7 cells/ml. The suspended cells, 10^6 cells/0.1 ml, were inoculated s.c. into the dorsal thoracic region in each of 4- to 5-week-old female nude mice (Simonsen, Gilroy, CA) under anesthesia with i.p. sodium pentobarbital (60 mg/kg). The animal protocol was approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis.

Paclitaxel treatment

Administration of vehicle or paclitaxel was initiated when a tumor became palpable with a linear size of approximately 0.2–0.4 cm 1 week after inoculation. Paclitaxel (Taxol) (Bristol-Myers/Squibb, Princeton, NJ), supplied at a concentration of 6 mg/ml in polyoxyethylated castor oil (Cremaphor EL) and ethanol (50/50%: v/v), was diluted with NS to provide a solution of 1.2 mg/ml. The vehicle consisted of polyoxyethylated castor oil (Sigma, St Louis, MO)/ethanol/NS (10/10/80%: v/v/v). The limitations of solubility and the large volume of drug solution necessitated i.p. administration of paclitaxel in mice as previously described [18–22]. A dose-range finding experiment revealed that two doses of paclitaxel, each given at 60 mg/kg 21 days apart, were optimal in inhibiting angiogenesis and growth in this tumor model [17]. Mice were divided into three groups with five animals each as follows: a control group was treated i.p. with the vehicle, at a volume identical to 20 mg/kg of paclitaxel weekly or at a volume identical to 60 mg/kg every 3 weeks for 6 weeks (Group A), a 7-day paclitaxel group was treated i.p. weekly with paclitaxel, 20 mg/kg, for six doses (Group B) and a 21-day paclitaxel group was treated with paclitaxel, 60 mg/kg, on days 0 and 21 for two doses (Group C). Tumor size was measured two-dimensionally every 2 days. On day 38, all animals were sacrificed by euthanasia with carbon dioxide. The experiments were repeated once. Tumors were removed, stored frozen in liquid nitrogen or fixed in buffered formalin and embedded in paraffin. The paraffin-embedded tumors were sectioned, stained with hematoxylin & eosin and examined under a light microscope for morphologic changes including the presence of necrosis.

Measurement of angiogenesis

Extent of angiogenesis was assessed by quantifying intratumoral microvasculature using confocal laser scanning microscopy as previously described [17]. Each frozen tumor was cut into 50-μm sections on glass slides and fixed in acetone. Microvasculature within the tumor was detected by a rat anti-mouse CD-31 monoclonal antibody (PharMingen, San Diego, CA), diluted 1:100 in PBS, followed by a FITC-conjugated polyclonal goat anti-rat IgG antibody (PharMingen) in 1:50 dilution in PBS. The FITC-labeled tissues were scanned with a Zeiss model LSM510 computer-assisted confocal laser scanning microscope equipped with an argon laser (Zeiss, Jena, Germany). Tissue sections were scanned at $\times 200$ magnification and an excitation wavelength of 488 nm. Using the z-stack function, a composite image was created from nine serial 6-μm fluorescent images acquired vertically for each microscopic field. Each image was analyzed using the LSM510 Software 3D (Zeiss). The total area of fluorescence (mm^2) in a microscopic field was integrated automatically by the computer, which was a quantitative index of angiogenesis as previously described [17].

Assessment of bone marrow suppression

Whole blood was drawn into a capillary tube from the tail vein of each mouse on days 0, 7, 14 and 21. The red blood cells were lysed, and the total white blood cell (WBC) count was determined using a hemocytometer and light microscopy [23].

Statistical analysis

Data are presented as mean ± SD. Significant differences ($p < 0.05$) of tumor growth rate, microvessel area and WBC count among the three treatment groups were determined by analysis of variance and the Bonferroni–Dunn test using the StatView software programs (SAS Institute, Cary, NC).

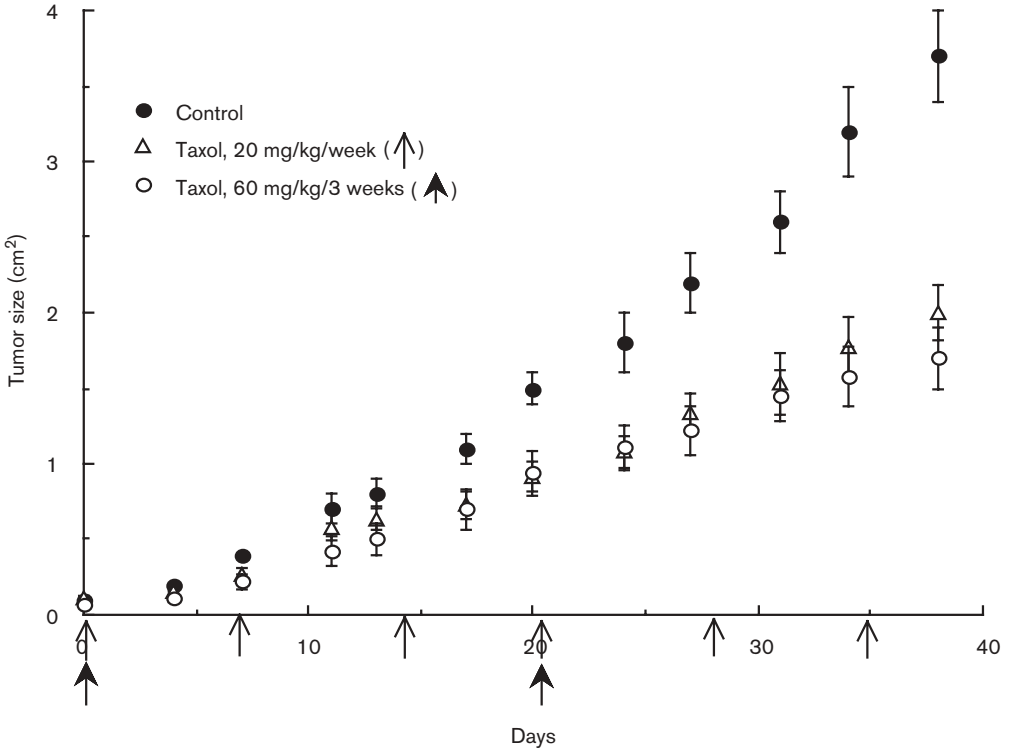
Results

After inoculation of A549 cells, tumors were generally palpable in animals within 7 days. The growth of tumors in each group of nude mice over the 38-day period is shown in Figure 1. In the control group, the tumors grew steadily at a rate of 0.06 cm^2/day for the first 2 weeks and more rapidly at 0.12 cm^2/day for the remaining 21-day period. The growth rate of tumors in both paclitaxel-treated groups grew more slowly than that of the control group for the first 2 weeks, although the difference was not statistically significant. However, the growth rate of tumors in both the paclitaxel-treated groups was significantly lower than the control group, at a rate of 0.05 cm^2/day for the remaining period ($p < 0.05$). There was no significant difference in the tumor growth rate between the animals treated with paclitaxel on the 7-day and 21-day schedules ($p < 0.05$).

Under light microscopy, tumors in each group of animals displayed glandular arrangement of malignant cells typical of an adenocarcinoma. Intratumoral necrosis was not observed in any animal group (Fig. 2a–c).

With confocal laser scanning microscopy, a complex network of blood vessels was observed within the tumors in the control mice (Fig. 3a). The index of angiogenesis in the control tumors was 0.07 ± 0.02 μm^2. For tumors in both groups of mice treated with paclitaxel, the network of microvessels was fragmented and irregular as shown in Figure 3(b and c), with an angiogenesis index of

Fig. 1



Time courses of growth of A549 lung cancer in three groups of nude mice after treatment with vehicle or with two schedules of paclitaxel (mean + SD, $n = 5$).

$0.03 \pm 0.02\,\mu m^2$ in each group. This angiogenesis index was significantly different from that of the control mice ($p < 0.05$).

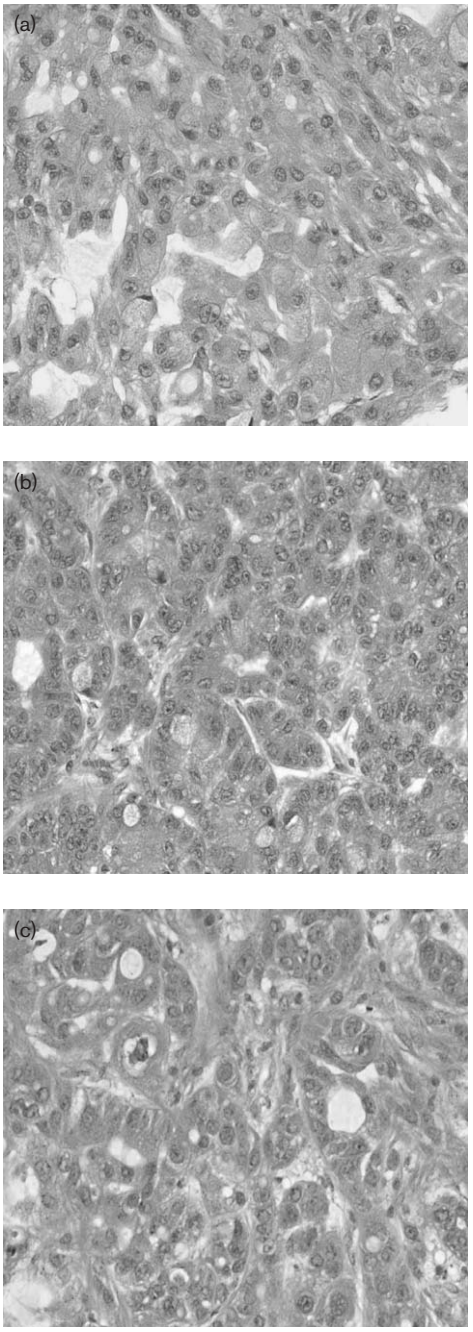
The time-courses of WBC counts following administration of vehicle or paclitaxel in the three groups of animals are shown in Figure 4. For mice treated on the weekly schedule, the WBC count remained stable and was similar to that of control over the 21-day period. However, for mice treated with paclitaxel on the 3-week schedule, the WBC count declined by 50% on day 7 and day 14, but it recovered to baseline by day 21. At day 14, the WBC count of the mice treated on the 3-week schedule was significantly lower than that of the control or the weekly-treated group ($p < 0.05$).

Discussion

It has been shown that frequent low-dose schedules improved antiangiogenic and anticancer efficacy of cyclophosphamide in experimental drug-resistant cancer models [7]. However, controversy remains regarding the relationship between scheduling of paclitaxel and its anticancer and antiangiogenic effects. In the clinical setting, it is feasible to conduct randomized trials to compare tumor responses and survival of patients treated with two different schedules of paclitaxel [8–10]. Nevertheless, it is difficult, if not impossible, to directly assess the antiangiogenic effect of paclitaxel within the context of clinical trials. With this limitation in mind, we applied an established model of human lung cancer and a method of confocal laser scanning microscopy to address the question of antiangiogenic and anticancer efficacy of two schedules of paclitaxel. The schedules of 7- and 21-day intervals employed in this study resemble that commonly used in clinical practice. Furthermore, the growth rate of this lung cancer model was appropriate for studying responses to paclitaxel over the study period.

Paclitaxel was administered i.p. in this study due to its low solubility, which limits its maximal concentration to 1.2 mg/ml. Thus, the volume of drug, required to achieve a dose of 20–60 mg/kg in mice, ranged from 0.33 to 1 ml. This volume of drug solution was not feasible for i.v. infusion in a mouse weighing only 20 g. Intraperitoneal paclitaxel has been shown to be bioavailable systemically in humans and rodents. Following a single i.p. administration in human, peak plasma levels were typically achieved by 1 h, which was believed to exceed the minimal concentration required to induce pertinent biological effects *in vitro* [21]. After a single i.p. dose of paclitaxel in rodents, plasma drug concentrations peaked at 3 h and were detectable for at least 24 h [22].

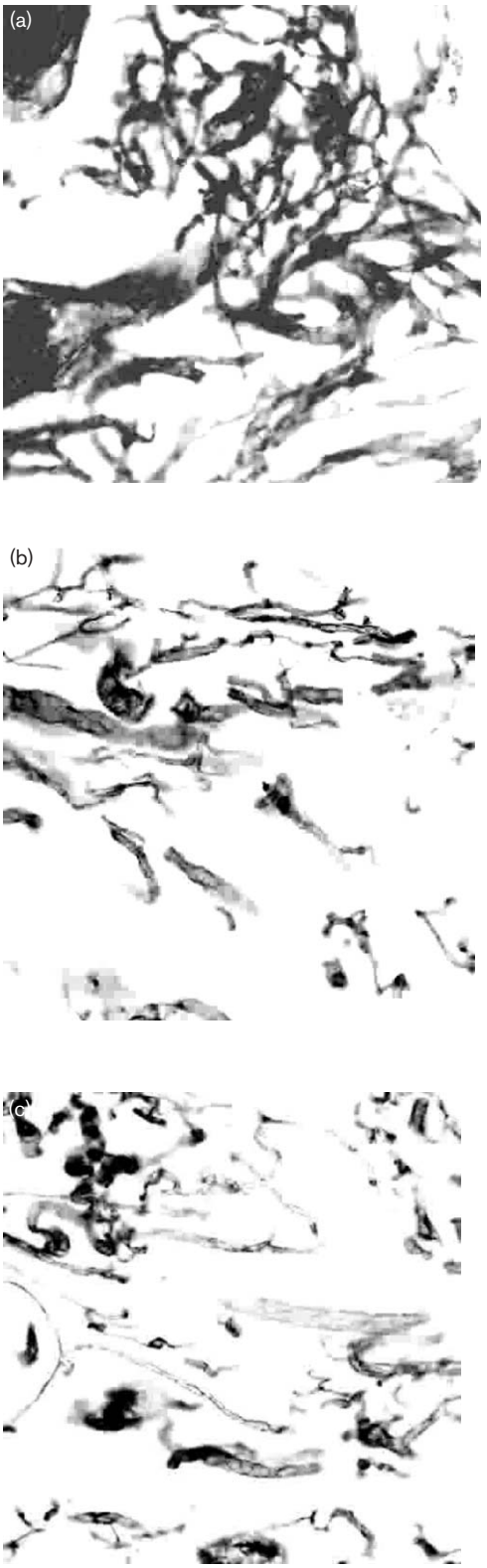
Fig. 2



Histology of the A549 lung cancer. (a) Mice treated with vehicle. (b) Mice treated with paclitaxel, 20 mg/kg/week. (c) Mice treated with paclitaxel, 60 mg/kg/3 weeks. (Magnification × 400).

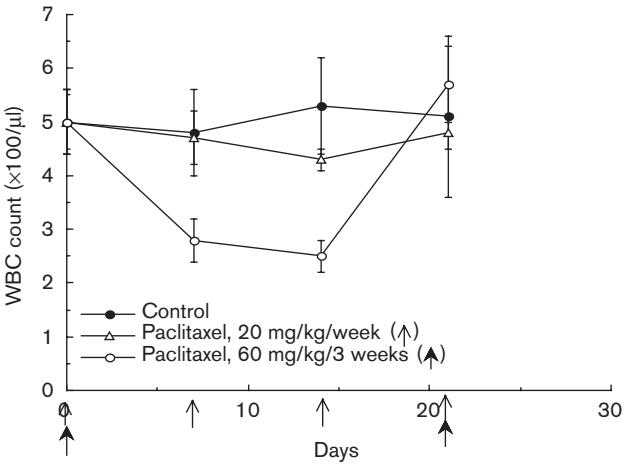
Conversely, paclitaxel was not detectable by 24 h in the peritoneal fluid. Therefore, the pharmacokinetics of i.p. paclitaxel resemble that of a prolonged intravenous infusion. This consideration is supported by reports of systemic anticancer activity of paclitaxel administered intraperitoneally either alone or in combination with other drugs [18–20].

Fig. 3



Microvessels of A549 lung cancer detected as CD31 immunofluorescence with confocal laser scanning microscopy. (a) Mice treated with vehicle. (b) Mice treated with paclitaxel, 20 mg/kg/week. (c) Mice treated with paclitaxel, 60 mg/kg/3 weeks. (Magnification × 200).

Fig. 4



Time courses of WBC counts in three groups of nude mice after treatment with vehicle or with two schedules of paclitaxel (mean + SD, $n = 5$).

In this study, we demonstrated that the efficacy of paclitaxel in retarding cancer growth was similar between the weekly treatment and the tri-weekly treatment groups. This was perhaps not surprising, as the dose intensity was identical between the two groups. For antiangiogenic activity, similarly, there was no statistical difference between the two schedules of paclitaxel in inhibiting the extent of microvasculature in this A549 lung cancer model. These findings are in contrast to the enhanced antiangiogenic potency of cyclophosphamide and 5-fluorouracil when these drugs were administered at low doses frequently or continuously in a mouse model of basic fibroblast growth factor-induced corneal neovascularization as reported by Browder *et al.* [7].

Supporting the applicability of this model to the clinical setting is the observation that paclitaxel given every 3 weeks caused significant leukopenia as compared to that of weekly dosing. This finding is similar to human clinical trials, where weekly paclitaxel was compared with tri-weekly paclitaxel in combination with carboplatin [8–10].

In conclusion, paclitaxel, administered weekly as compared to tri-weekly, exhibits similar anticancer and antiangiogenic activities, but causes less leukopenia in a lung cancer model. This study supports the use of weekly paclitaxel in clinical practice, and supports further investigation of the antiangiogenic properties of paclitaxel.

References

1 Sledge GW, Neuberg D, Bernardo P, Ingle JN, Martino S, Rowinsky EK, *et al.* Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: an intergroup trial (E1193). *J Clin Oncol* 2003; **21**:588–592.
2 Kelly K, Crowley J, Bunn Jr PA, Presant CA, Grevstad PK, Moinpour CM, *et al.* Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: a Southwest Oncology Group trial. *J Clin Oncol* 2001; **19**:3210–3218.
3 Markman M, Liu PY, Wilczynski S, Monk B, Copeland LJ, Alvarez RD, *et al.* Phase III randomized trial of 12 versus 3 months of maintenance paclitaxel in patients with advanced ovarian cancer after complete response to platinum and paclitaxel-based chemotherapy: a Southwest Oncology Group and Gynecologic Oncology Group trial. *J Clin Oncol* 2003; **21**:2460–2465.
4 DeVore RF, Jagasia M, Johnson D. Paclitaxel by either 1-hour or 24-hour infusion in combination with carboplatin in advanced non-small cell lung cancer: preliminary results comparing sequential phase II trials. *Semin Oncol* 1997; **24**(**suppl**):27–29.
5 Akerly W, Sikov WM, Cummings F, Safran H, Strenger R, Marchant D. Weekly high-dose paclitaxel in metastatic and locally advanced breast cancer: a preliminary report. *Semin Oncol* 1997; **24**(**suppl**):87–90.
6 Chang AY, Boros L, Asbury R, Hui L, Rubins J. Dose-escalation study of weekly 1-hour paclitaxel administration in patients with refractory cancer. *Semin Oncol* 1997; **24**(**suppl**):69–71.
7 Browder T, Butterfield CE, Kraaling BM, Shi B, Marshall B, O'Reilly MS, *et al.* Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res* 2000; **60**:1878–1886.
8 Ukena D, Schroeder M, Dittrich I, Guschall WR, Ko YD, Saar T, *et al.* Phase III randomized trial comparing paclitaxel/carboplatin every 3 weeks with weekly paclitaxel/carboplatin in patients with advanced non-small-cell lung cancer. *Proc Am Soc Clin Oncol* 2003; **22**:623.
9 Belani CP, Barstis J, Perry MC, La Rocca RV, Nattam SR, Rinaldi D, *et al.* Multicenter, randomized trial for stage IIIB or IV non-small-cell lung cancer using weekly paclitaxel and carboplatin followed by maintenance weekly paclitaxel or observation. *J Clin Oncol* 2003; **21**:2933–2939.
10 Goble S, Bear HD. Emerging role of taxanes in adjuvant and neoadjuvant therapy for breast cancer: the potential and the questions. *Surg Clin North Am* 2003; **83**:943–971.
11 Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly *in vitro* by taxol. *Nature* 1979; **22**:665–667.
12 Rowinsky EK, Donnehower RC, Jones RJ. Microtubule changes and cytotoxicity in leukemic cell lines treated with taxol. *Cancer Res* 1988; **48**:4093–4100.
13 Belotti D, Nicoletti I, Vergani V, Rieppi M, Drudis T, Viale G, *et al.* Paclitaxel (Taxol), a microtubule affecting drug, inhibits tumor induced angiogenesis. *Proc Am Ass Cancer Res* 1996; **37**:397.
14 Oktaba AMC, Hunter WL, Arsenault AL. Taxol: a potent inhibitor of normal and tumor-induced angiogenesis. *Proc Am Ass Cancer Res* 1995; **16**:454.
15 Klauber N, Parangi S, Flynn E, Hamel E, D'Amato R J. Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. *Cancer Res* 1997; **57**:81–86.
16 Lau DH, Xue L, Young LJ, Burke PA, Cheung AT. Paclitaxel (Taxol): an inhibitor of angiogenesis in a highly vascularized transgenic breast cancer. *Cancer Biother Radiopharm* 1999; **14**:31–36.
17 Guo LL, Burke P, Lo SH, Gandour-Edwards R, Lau D. Quantitative analysis of angiogenesis using confocal laser microscopy. *Angiogenesis* 2001; **4**:187–191.
18 Shi B, Yaremko B, Haijian G, Terracina G, Bishop WR, Liu M, *et al.* The farnesyl protein transferase inhibitor SCH66336 synergizes with taxanes *in vitro* and enhances their antitumor activity *in vivo*. *Cancer Chemother Pharmacol* 2000; **46**:387–393.
19 Papadopoulou MV, Ji M, Ji X, Bloomer WD, Hollingshead MG. Therapeutic advantages from combining paclitaxel with the hypoxia-selective cytotoxin NLCQ-1 in murine tumor- or human xenograft-bearing mice. *Cancer Chemother Pharmacol* 2002; **50**:501–508.
20 Nguyen DM, Lorang D, Chen A, Stewart JH, Tabibi E, Schrump DS. Enhancement of paclitaxel-mediated cytotoxicity in lung cancer cells by 17-allylamino geldanamycin: *in vitro* and *in vivo* analysis. *Ann Thorac Surg* 2001; **72**:371–379.
21 Rowinsky EK. Clinical pharamacology of taxol. *J Natl Cancer Inst Monogr* 1993; **15**:25–38.
22 Mohamed F, Marchettini P, Stuart OA, Sugarbaker PH. Pharmacokinetics and tissue distribution of intraperitoneal paclitaxel with different carrier solutions. *Cancer Chemother Pharmacol* 2003; **52**:405–410.
23 Barker K. *At the Bench. A Laboratory Navigator.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1998, pp. 225–226.